

Building better T-cell-inducing malaria vaccines

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Summary

Since malaria continues to account for millions of deaths annually in endemic regions, the development of an effective vaccine remains highly desirable. The life cycle of malaria poses a number of challenges to the immune response since phases of the cycle express varying antigen profiles and have different locations, thus requiring differing antigenic targets and effector mechanisms. To confer sterile immunity, a vaccine would have to target the pre-erythrocytic stages of infection. Since at this stage the parasite is hidden within liver cells, the host defence predominantly requires cell-mediated immunity, chiefly T cells, to eliminate infected hepatocytes. The development of such vaccines has progressed from irradiated sporozoites, through recombinant proteins, to recombinant DNA and viral vectors. Some of the experimental vaccination regimens that explore various combinations of vaccines for priming and boosting, together with numbers of vaccinations, interval between them, and the vaccination site, are revealing strong immunogenicity and evidence of efficacy in human challenge studies and in field trials. Such approaches should lead to deployable vaccines that protect against malarial disease.

Keywords: malaria; vaccines; T cell

Introduction

Out of the 500 million annual cases of malaria, the infection continues to kill 1–2 million people a year in endemic regions, almost 1 million of whom are children under 5 years,¹ despite the availability of modern drugs and mosquito-killing insecticides. Drug-resistant parasites, the increasing costs of effective treatment and the poor uptake of a cheap but effective measure (insecticide-treated bed nets) make efforts to develop a vaccine imperative to attempt to reduce the disease burden. However, malaria vaccine development is a complicated and lengthy process. Along with locational differences within human tissues of the parasite during its life cycle, malaria also has differing patterns of antigen expression. Thus, immune responses need to be of the appropriate type and targeted against a relevant antigen, as well as being of sufficient magnitude and present at the correct time and location. In addition, it is desirable that immune responses are effective for long periods after the vaccination process and protect against multiple strains of malaria. Cell-mediated immune responses, mainly CD4⁺ and

CD8⁺ T cells, are implicated in protection against the liver stage of infection² and antibodies are involved in the protection against sporozoites. Vaccines that are highly effective against either or both of these life-cycle stages should produce sterile immunity. The association between possession of the HLA-B*5301 major histocompatibility complex (MHC) class I allele and protection from severe forms of *Plasmodium falciparum* malaria infection in African children lends weight to a role of CD8 T-cell responses in humans in endemic areas,³ together with the CD8 T-cell-mediated protection seen in rodents following immunization with irradiated sporozoites.⁴ Thus, pre-erythrocytic immunity has, to varying degrees, been elicited using vaccines comprising irradiated sporozoites, recombinant protein antigens and, more recently, antigen-encoding recombinant DNA and viruses. Modifications to the molecular make-up of vaccines, their combinations during sequential immunization, and the choice of antigen, together with detailed analysis of immune responses elicited and infection challenge studies may well lead to vaccination regimens that considerably reduce the parasite burden at the liver stage, eventually

Abbreviations: AMA, apical membrane antigen; Exp, exported; GLURP, glutamate rich protein.

conferring protective immunity on those who need it. This article aims to expand on these concepts, but without unduly duplicating excellent previously published reviews,^{5–7} and will attempt to present an up to date immunological perspective on this form of anti-malarial vaccine.

Biology of *Plasmodium falciparum*

Some knowledge of the biology and life cycle of *P. falciparum* (simplified in Fig. 1) is required if the design of malaria vaccines is to be appreciated. During the blood meal of an infected female anopheline mosquito [(a) in Fig. 1] 5–20 sporozoites are injected from the fly's salivary glands; they enter the bloodstream and rapidly invade hepatocytes within 30 min to 1 hr [(b) in Fig. 1]. Sporozoites are known to express several surface proteins, two of which are the highly expressed antigens; circumsporozoite (CS) protein and thrombospondin-related adhesion protein (TRAP). Being well characterized, and the targets of protective immune responses in humans as well as rodents (as described below), these antigens are considered to be major vaccine candidates. Once within the hepatocytes additional antigens are expressed, including liver stage antigen-1 (LSA-1) and LSA-3, and exported (Exp)-1. It takes about 1 week for the development of merozoites, typically 20 000–40 000 per original sporozoite, which are released into the bloodstream [(c) in Fig. 1] following the rupture of hepatocytes. Merozoites,

which express a range of blood-stage antigens that are largely different from those of sporozoites, e.g. merozoite surface proteins (MSP) -1, -2 and -3, apical membrane antigen (AMA)-1 and glutamate-rich protein (GLURP), invade red blood cells [(d) in Fig. 1], replicate, and cause the red blood cells to rupture, thus releasing more merozoites. After several blood-stage cycles a proportion of merozoites differentiate into male and female gametocytes which, if ingested by mosquitoes [(e) in Fig. 1] during a blood meal, form oocysts within the mosquito gut that give rise to sporozoites capable of infecting a new host. Although the blood stage of infection may lead to a serious illness, and in some cases death, of the host, clinical immunity develops after repeated exposure and not only protects against severe forms of the disease but eventually reduces the level of parasitaemia. However, sterile immunity indicating protection against re-infection is hardly ever seen. The objective of vaccination against the liver stage is to induce, especially in young children who are most at risk, either sterile immunity or a sufficient reduction in parasite numbers reaching the blood-stage to attenuate disease. The latter effect would also provide the opportunity for beneficial natural immunity to develop.

Immunization against malaria

Rational malaria vaccine design must be firmly rooted within an immunological basis incorporating aspects of initiating (priming) and expanding (boosting) responses

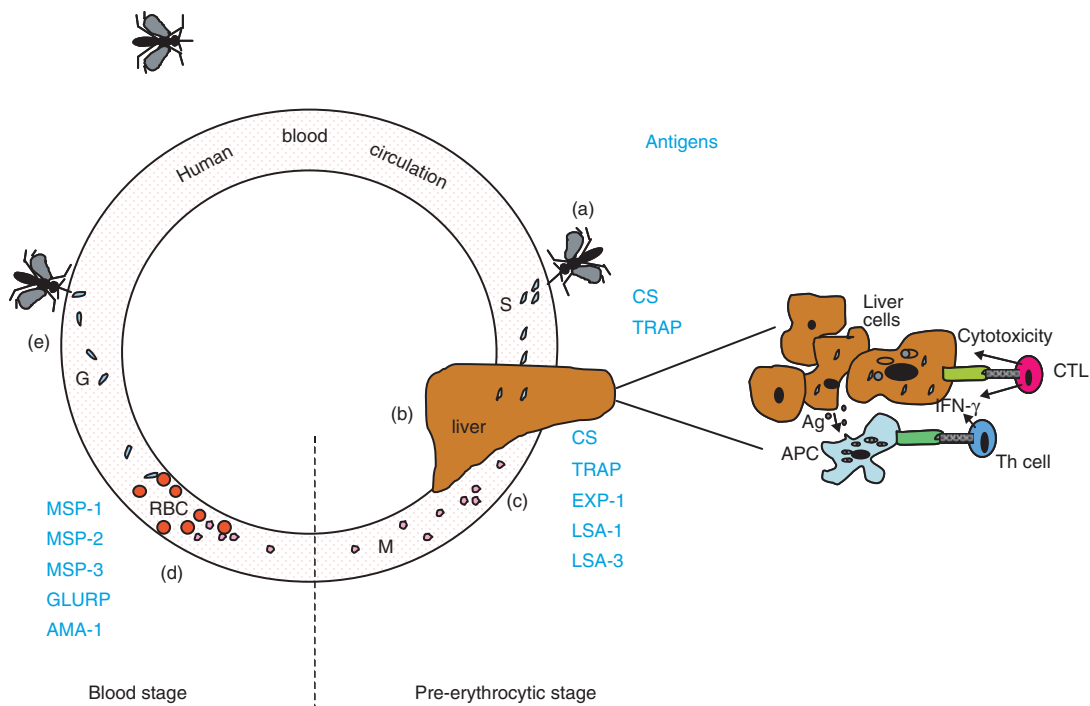


Figure 1. Malaria life cycle showing antigens expressed and a representation of chief liver-phase immune effector mechanisms. S, sporozoites; M, merozoites; G, gametocytes.

of the appropriate type and specificity. Thus, in the case of vaccines against pre-erythrocytic stages, antibodies against the CS antigen may 'neutralize' sporozoites by blocking or eliminating them from circulation and thus preventing hepatocyte infection. Antibody-generating vaccines against infectious organisms typically comprise attenuated organisms or recombinant subunit antigens, together with an adjuvant providing a slow-release depot possibly with some stimulus of innate immunity to initiate the immune response. CD4⁺ T-helper cells must also be generated because they are required to activate and fine-tune the B cells during priming and boosting vaccinations to produce antibodies of appropriate avidity and subtype and of sufficient magnitude.

However, once hepatocytes are infected during the normal malarial life cycle these cells must be specifically targeted by T cells in a site, the liver, that is believed to be relatively immunosuppressive in nature. Indeed, the liver sinusoidal endothelial cells are believed to induce T-cell tolerance through their presentation of local antigens.⁸ Furthermore, vaccines will be chiefly deployed in regions where individuals are already infected. Red blood cells parasitized with *P. falciparum* have been shown to suppress dendritic cell activity,⁹ may cause apoptosis of parasite-specific T cells¹⁰ and cause secretion of immune-suppressive transforming growth factor- β .¹¹

There is evidence that the main anti-parasite effector mechanism in the liver is interferon- γ (IFN- γ) produced mainly by CS-specific CD8⁺ and CD4⁺ T cells that inhibit parasite development within hepatocytes.^{2,7,12–15} CD8⁺ cytolytic T lymphocytes (CTL) that are capable of recognizing malarial antigens presented by MHC class I molecules on the surface of infected hepatocytes may also play a role (Fig. 1b). Whilst such CTLs may kill the hepatocytes through pore-forming perforin proteins, which allow apoptosis-inducing granzymes to enter and kill the target cells, immunity can also be achieved in perforin knockout mice immunized with irradiated sporozoites.¹⁶ IFN- γ production may enhance cytolytic mechanisms by increasing class I molecules on the surface of hepatocytes, making those hepatocytes better targets for CTL lysis, and activating subsidiary effector cells such as natural killer cells and macrophages, which are also able to kill the target cells through CTL-like mechanisms, tumour necrosis factor- α induction and nitric oxide production.

The efficient priming of CTLs requires, during vaccination, the introduction of liver-stage malarial antigens into the endogenous (intracellular) pathway of antigen processing and subsequent presentation via MHC class I on professional antigen-presenting cells (APC), and this can be achieved using recombinant vectors that encode the appropriate malarial antigens. The use of recombinant DNA vectors or viruses requires a sequence of priming and boosting immunizations to achieve an optimal and sufficient level of effective immunity (Fig. 2). Since anti-

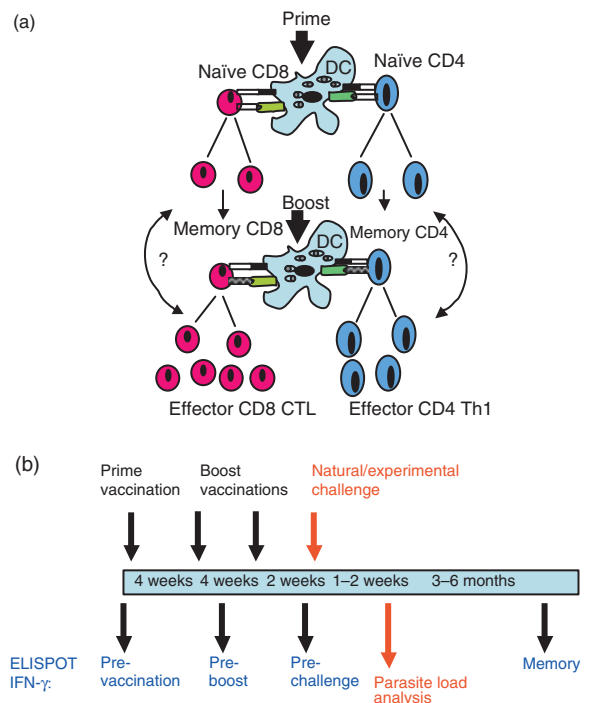


Figure 2. Prime-boost concept and typical trial protocol. The prime-boost concept (a) comprises the generation of antigen-specific memory T cells during the prime vaccination with one vector type followed at a later time point by a boost with a second vector type which expands/differentiates memory to effector T cells. Qualitative differences in the immunization processes during prime and boost, giving rise to differing T-cell responses, may occur at the level of the APC (DC). A typical vaccination protocol (b) involves usually two prime vaccinations followed by a heterologous boost, with regular immune monitoring (chiefly ELISPOT). Challenge occurs 2–4 weeks later.

vector immunity may depress immunity against the vectored antigen, the sequential delivery of differing vectors, heterologous prime-boosting, is being pursued. Anti-vector immunity may be in the form of anti-vector antibodies that neutralize the vector or vector-specific T cells that compete for MHC binding or growth factors with vectored-antigen-specific T cells, or even that kill APCs. Additionally, particular vectors are known to be better or worse at priming and boosting T-cell responses, as described in the next section. The generation of CTL usually requires assistance from cognate antigen-specific CD4⁺ T helper cells. These T cells will recognize malarial antigens presented, via the exogenous antigen-processing pathway, by MHC class II molecules on APC. The APC becomes 'empowered' by CD40 ligation to prime and activate CD8⁺ CTLs,¹⁷ particularly memory cells.¹⁸ CD4⁺ T cells may also help in effector processes by producing IFN- γ , when they recognize antigen presented by local APC, and this IFN- γ activates subsidiary cells, as with CD8⁺ cells.

The heterologous prime-boost approach has been shown to induce protective immunity against malaria in

rodents challenged with the lethal strain of *P. yoelii*^{19,20} initially using replicating viral vectors. It has been shown that priming with non-replicating plasmid DNA or adenovirus encoding the *P. berghei* pre-erythrocytic antigens CS (PbCS) and/or TRAP, followed by boosting with non-replicating recombinant modified vaccinia virus Ankara (MVA) induced complete or almost complete protection which correlated with CD8⁺ T-cell responses.^{21–23} Using the attenuated avian poxvirus FP9 (an attenuated strain of fowlpox virus), as the priming agent instead of DNA, the immunogenicity and level of protection in mice could be increased even further.²⁴

For measuring immune responses following vaccination, the technique of IFN- γ ELISPOT has proved very useful. This assay is able to give absolute numbers of antigen-specific IFN- γ -secreting cells (CD8⁺ and CD4⁺) in a rapid (18 hr) assay involving cytokine capture on a cellulose membrane and antibody-mediated colour development revealing a spot for every cytokine-secreting cell. Modifications to this assay involving more extensive culture are also helping to reveal longer lasting memory T-cell responses.

Protective T-cell responses against the blood stage of infection have been demonstrated following low-dose vaccination with *P. falciparum*-infected red blood cells²⁵ or recombinant antigens such as MSP-1.²⁶ However, it is thought that such immunity may also lead to immune-mediated pathology and, to be beneficial, may need to be balanced by anti-inflammatory responses.²⁷

Pre-erythrocytic vaccine studies in humans

The first demonstration of protection from malaria by vaccination in humans, over 30 years ago, involved the inoculation of subjects with sporozoites through multiple bites with irradiated mosquitoes²⁸ and subsequent studies showed that such immunizations elicited CTLs.²⁹ Although the use of irradiated sporozoites is not generally thought to be feasible for widespread vaccination, such studies gave considerable impetus to the development of more widely applicable vaccines. Thus the aim over recent years has been to develop subunit vaccines where candidate antigens are genetically cloned and expressed either in the laboratory and purified for administration, or recombinant genes are transferred via vectors to the vaccinee where antigens are expressed by the host tissues. Antigens in the form of purified proteins may often be poorly immunogenic, particularly for antigen-specific IFN- γ production and eliciting CD8⁺ CTLs, which are believed to be a key mechanism for the elimination of hepatocytes harbouring malaria. Thus, ways of enhancing immunogenicity are pursued such as the use of adjuvants or stimulatory vectors. The current lead vaccine candidate of the recombinant protein type is known as RTS,S, based on the major pre-erythrocytic stage antigen CS.³⁰ To produce the RTS construct, DNA encoding hepatitis B surface (HBs) anti-

gen was fused to the C-terminal half of the *P. falciparum* CS protein, containing T-cell epitopes, and to the NANP epitope repeats that are known to act as B-cell epitopes. When expressed in yeast cells together with further hepatitis B subunits, the HBs subunit binds to RTS to form RTS,S particles. To formulate the vaccine, these particles are further mixed with AS02 adjuvant (comprising monophosphoryl lipid A, the saponin component QS21 and an oil-in-water emulsion) and then administered in two or three doses intramuscularly. Trials on non-immune volunteers (in non-endemic regions) revealed the generation of high titres of antibody against CS and about 40% protection in the sporozoite challenge model using the homologous 3D7 *P. falciparum* strain via mosquito bites.³¹ Field trials in naturally infected Gambian adults also showed significant protection. During the first 9 weeks that followed the third dose the vaccine offered 71% efficacy; however, this did not last. During the following 6 weeks efficacy was reduced to 0%.³² It is important to note that low-level immune responses to pre-erythrocytic malarial antigens already exist in people in malaria-endemic regions, in particular to CS³³ and TRAP,³⁴ and so responses following vaccination may differ from and be higher than those of non-immune individuals. Whilst cell-mediated immunity was observed with RTS,S³⁵ a recent study identified IFN- γ -secreting CD4⁺ T cells in RTS,S-vaccinated and non-vaccinated subjects specific for a conserved epitope that correlated with protection from *P. falciparum* infection and disease in the field in the Gambia.³³ These cells needed to be cultured before they could be measured by standard ELISPOT, underlining the importance of being able to measure memory cells as well as effector cells during vaccine studies. Following successful safety trials in young children in Mozambique, the Malaria Vaccine Initiative in conjunction with Glaxo-SmithKline Biologicals very recently showed in some 2000 RTS,S-vaccinated children (aged 1–4 years) a 30% reduction in the rate of development of clinical malaria with some evidence of a greater reduction in incidence of severe malaria.³⁶ A vaccine similar to RTS,S called ICC-1132, based on hepatitis B core antigen fused to fragments of CS, has also entered trials following successful preclinical studies.³⁷ In mice and rhesus monkeys, ICC-1132, formulated in an oil-based adjuvant, ISA Seppic 720, resulted in anti-NANP antibodies exceeding 1 in 10⁶, remaining at a high level for up to a year³⁶ (Dubovsky *et al.*, unpublished abstract from the 5th Annual Novel Adjuvant Meeting WHO/TDR Annecy France, June 2003). Importantly, a second dose given 8 weeks later did not significantly increase the antibody levels. Based on this observation a single dose of 50 μ g of this vaccine was given to human volunteers. However, this induced only moderate levels of anti-NANP antibodies, and the subsequent sporozoite challenge revealed no evidence of protection for this single-dose regime.³⁸ Immunization with recombinant LSA-3 showed

protection from *P. falciparum* challenge in chimpanzees.³⁹ Vaccines comprising synthetic peptides incorporating B-cell and CD4 and CD8 T-cell epitopes from *P. falciparum*, formulated with alum or QS21, have demonstrated immunogenicity⁴⁰ and warrant further trial studies.

Recombinant DNA-based vaccines are currently offering a promising approach to vaccination, the most immunogenic contributions of vectors of which were first established in animal models, as described earlier. In general, naked DNA and viruses such as attenuated fowlpox (FP9) or adenovirus have been found to be preferable for priming, whilst viruses such as MVA are better at boosting the response. Such results have been achieved using vectored antigens such as the pre-erythrocytic antigen TRAP, responses to the latter being further supplemented by encoding additional T- and B-cell epitopes from other malarial antigens [encoded in a multi-epitope (ME) string]. Immunization regimens using this vaccine, ME-TRAP, comprising DNA-vectored followed by MVA-vectored antigen by various combinations and routes, as well as showing highly satisfactory safety profiles,⁴¹ elicited unprecedented levels of IFN- γ -secreting T cells, and resulted in a significant delay of on average 40 hr in time to parasitaemia upon sporozoite challenge.⁴² Although no complete protection was reported, such a delay in the occurrence of parasites in the peripheral blood as compared to that in non-vaccinated controls indicates a reduction of the parasite burden in the liver of about 80%.⁴³ Cell depletion and intracellular cytokine fluorescence-activated cell sorter analysis indicate that the former regime generated mainly CD4⁺ IFN- γ -secreting cells.⁴⁴

An ME-TRAP regime has been assessed for protective efficacy in a large field trial in the Gambia. Two doses of 2 mg DNA encoding ME-TRAP, given 3 weeks apart, followed by MVA ME-TRAP (1.5×10^8 plaque-forming units) given 3 weeks later prior to the start of the rainy season, was administered to 296 adult males who were compared to controls in rural Gambia.⁴⁵ Despite inducing good immunogenicity that could be reboosted a year later,⁴⁶ the regime failed to induce more than 10% protective efficacy against infection, a non-significant level.

When recombinant FP9 was used for priming, followed by MVA, expressing the ME-TRAP antigen, complete protection of two subjects was observed in Oxford, alongside significant delay in parasitaemia for the remaining subjects (Webster *et al.* submitted for publication). Importantly, there was evidence of protection lasting up to 20 months in this study. This latter regimen induced good numbers of CD8⁺ as well as CD4⁺ T cells secreting IFN- γ .⁴⁴ Indeed, a cultured ELISPOT approach which allows the expansion and/or differentiation of memory T cells into IFN- γ -secreting effector cells has revealed memory T-cell responses many months after vaccination that may correlate with delay to parasitaemia (Keating *et al.* submitted for publication). This further characteri-

zation of the responding T cells in terms of effector and memory phenotype appears to be important in determining immune correlates of protection.

A series of trials is currently investigating recombinant MVA, FP9 and plasmid DNA expressing the full-length CS protein in various combinations, with phase I and II trials performed in Oxford as well as in endemic areas. Recently, a six-antigen polyprotein, comprising both liver-stage and blood-stage antigens encoded by poxviruses, was designed to elicit broad immune responses that may better combat plasmodial antigen escape mutants.⁴⁷ Following impressive preclinical data, clinical trials are planned to investigate the use of this vaccine. A vaccinia vaccine expressing multiple antigens from separate promoters showed some promise as a single agent in earlier trials.⁴⁸

Vaccination regimens comprising combinations of recombinant protein and vectored-antigen vaccines are being explored and may provide additional alternatives.⁴⁹

Very recently, malaria vaccine research has come full circle with a vaccine comprising genetically modified whole *P. berghei* sporozoites demonstrating complete protection in a murine sporozoite challenge model.⁵⁰ These immunizing sporozoites retained their ability to infect hepatocytes but were unable to establish blood-stage infection because of their deletion of an essential gene, UIS3. The issue of producing sufficient numbers of sporozoites for human vaccination remains.

Concluding remarks

A vaccine, or vaccination regime, capable of making an impact against malaria needs to be sufficiently immunogenic to inhibit or reduce parasitaemia whilst being safe to administer and affordable for target populations, mainly in Africa. Experimental trials, testing immunization regimes such as heterologous prime-boost, need to further optimize such regimens and, particularly, need to show immune correlates of protection. This can be a laborious process requiring thorough evaluation of many options and regimes, but current opinion suggests that a deployable vaccine could be available within the next decade.

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